a microparticle comprising an active agent and a glutamine-rich polymer having transglutaminase substrate reactive groups, wherein the transglutaminase substrate reactive groups are surface available.

124. The composition of claim 123, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of endogenous transglutaminase.

125. The composition of claim 123, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of exogenous transglutaminase.

135. (Amended) The composition of claim 123, wherein the glutamine-rich polymer is covalently linked to the synthetic polymer.

136. The composition of claim 123, wherein the glutamine-rich polymer comprises a polymer of amino acids and wherein at least 20% of the amino acids are glutamines.

143. (Amended) A composition comprising

a microparticle comprising a non-nucleic acid nonlabeling active agent, and

covalently attached surface available transglutaminase substrate reactive groups,

wherein the microparticle is 100 nm to 500 nm in size.

144. (Amended) The composition of claim 143, wherein the surface available transglutaminase substrate reactive groups are free pendant groups.

Claim Objection

The Examiner has objected to claim 135 because the claim is dependent on cancelled claim 131. Applicants have amended claim 135 to depend from claim 123, and respectfully request that the Examiner reconsider and withdraw the objection.

Remarks

The specification has been amended to correct typographical errors.

Claims 1, 22, 25, 26, 76, 135, 144 (as filed) and 145 (as filed) have been amended.

Claim 1 has been amended to clarify that the transglutaminase is endogenous to the skin surface, as suggested by the Examiner. Support for this amendment can be found on page 16, lines 12-13.

Claims 22 and 25 have been amended to clarify the group of polymers in the Markush group, as suggested by the Examiner. Support for these amendments can be found on page 18, lines 11-15 and 16-23.

Claims 26 and 76 have been amended to include the limitation that the microparticle is nonlabeling, meaning that the microparticle does not contain a labeling agent (i.e., an agent that is simply a passive label with no function other than being a label). Support for these amendments can be found on page 24, lines 9-10.

Claim 135 has been amended to depend from claim 123, a pending claim, rather than cancelled claim 131.

Originally pending claims 144 and 145 have been newly re-numbered as claims 143 and 144 in view of missing claim 138, as suggested by the Examiner. Newly re-numbered claim 144 has been further amended to depend from newly re-numbered claim 143.

Newly re-numbered claim 143 has been amended to include the limitation that the active agent is a nonlabeling active agent. A nonlabeling active agent is defined in the specification on page 24, lines 9-10 as an agent "that is not simply a passive label with no function, when applied to a body tissue, other than being a label."

Claims 1-26, 51, 75-77, 102, 117-119, 123-125, 135, 136, 143 and 144 are currently pending.

No new matter has been added.

Claim Fees

There are currently 7 independent claims and 41 total claims pending. Applicants previously paid for 7 independent claims (i.e., paid for an additional 4 independent claims over the filing fee allotment of 3) and 41 total claims (i.e., paid for an additional 21 total claims over the filing fee allotment of 20). No new claims fee is considered due in view of this amendment.

The Claimed Invention

The claimed invention provides methods and compositions comprising microparticles having surface available transglutaminase substrate reactive groups. These surface reactive groups are present in an amount sufficient to attach the microparticles to a body tissue such as a skin surface of a subject either in the presence of endogenous or exogenous transglutaminase. The reactive groups can be lysines or glutamines, and they can further be comprised in a polymer.

Rejection under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims 1-25, 21, 22, 24, 25, 102, 117-119, 123-125, 135 and 136 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-25

The Examiner has rejected claims 1-25 as confusing and unclear because of the recitation of "the presence of endogenous transglutaminase." The Examiner states that it is not clear what the transglutaminase is endogenous to.

Applicants have amended claim 1, as suggested by the Examiner, to recite that the skin surface contains endogenous transglutaminase. This amendment does not narrow the scope of the claim.

Claims 21, 24, 102, 117-119, 123-125, 135 and 136

The Examiner has rejected claims 21, 24, 102, 117-119, 123-125, 135 and 136 because of the recitation of "glutamine-rich" and "lysine-rich" since the Examiner states that "rich" is a relative and subjective term.

Applicants respectfully direct the Examiner to page 18, lines 16-21 which teach that

> "a polymer rich in glutamine or lysine is a molecule wherein at least 20% of the units of the polymer carry a carboxamide, an aliphatic amine, or both, such as glutamine, lysine or glutamine and lysine, or wherein the molecule includes at least 3, preferably 4 and most preferably 5 separate and discretely spaced by a regular distance carboxamides or aliphatic amines, such as occurs with contiguous, linked glutamines or lysines."

In view of this teaching, the terms "glutamine-rich" and "lysine-rich" are considered definite.

Claims 22 and 25

The Examiner has rejected claims 22 and 25 as being confusing and unclear because of the recitation of a polymer selected, according to the Examiner, from a group of materials that are not polymers. Although Applicants respectfully traverse the Examiner's characterization of the member of Markush group as not being polymers, Applicants have nonetheless amended claims 22 and 25 as suggested by the Examiner. This amendment does not narrow the scope of the claim.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1-25, 21, 22, 24, 25, 102, 117-119, 123-125, 135 and 136 under 35 U.S.C. §112, second paragraph.

Claims Free of the Prior Art

Applicants acknowledge the Examiner's finding that claims 22 and 25 are free of the prior art.

Rejection under 35 U.S.C. §102(b)

The Examiner has rejected claims 123-125, 143 and 144 under 35 U.S.C. §102(b), as being anticipated by Bernstein et al. (US 5,679,377) or Mathiowitz et al. (US 5,271,961). According to the Examiner, "the protein microspheres of Bernstein et al. or Mathiowitz et al. are inherently glutamine-rich, and inherently contain sufficient transglutaminase reactive substrate groups on their surfaces to attach the microspheres to skin in the presence of endogenous or exogenous transglutaminase as in claims 123-125." The Examiner further states that "crosslinking protein with transglutaminase as disclosed by Bernstein et al. or Mathiowitz et al. is optional and not essential ... (and) ... the microspheres of Bernstein et al. or Mathiowitz et al. can contain a non-nucleic acid active agent and have a particle size in the range of claims 143 and 144."

Applicants respectfully traverse the rejection. There is no evidence that, after manufacture, the microspheres taught by either Bernstein et al. or Mathiowitz et al. have surface available transglutaminase substrate reactive groups (i.e., present in an accessible form to transglutaminase), or that such groups are present in an amount sufficient to attach the microspheres to a body tissue such as a skin surface using either endogenous or exogenous transglutaminase.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. §102(b).

Rejection under 35 U.S.C. §103(a)

Rejection in view of Richardson et al., Bernstein et al., Mathiowitz et al. and Won

The Examiner has rejected claims 1, 3-19, 23, 24, 26, 51, 75-77, 123-125, 135, 136, 143 and 144 under 35 U.S.C. §103(a), as being unpatentable over Richardson et al. (U.S. 5,490,980) in view of Bernstein et al. (US 5,679,377) or Mathiowitz et al. (US 5,271,961) each taken with Won (US 5,145,675).

According to the Examiner, "Richardson et al. disclose attaching an active agent inherently containing or modified to contain an alkylamine ($R'NH_2$) group to skin, hair or nails by crosslinking the active agent through the alkylamine group to glutamine residues of skin, hair or nails ... (and) ... the active agent may be an intact protein."

According to the Examiner, "Bernstein et al. disclose protein microspheres that can be made of a prolamine protein containing a high number of hydrophobic amino acids such as glutamine ... (and) ... the microspheres can be formed entirely of protein or protein in combination with polymer ... the

microspheres can have a size of about 50 to 100 nm to about 20 microns, and preferably from about 100 nm to about 5 microns ... composite protein-polymer microspheres can be formed by combining the protein with a non-protein natural or synthetic polymer ... the composite microspheres may be the form of protein microspheres coated with a polymer or polymer microspheres coated with a protein ... the protein can be modified chemically or enzymatically to provide a property such as enhanced surface reactivity ... enhanced stability of the protein may be obtained by crosslinking the protein with transglutaminase ... the microspheres can be used for delivery of a biologically active agent such as a drug to provide a desired release rate at a targeted site ... (and) ... microspheres containing a desired compound can be topically applied to skin or other areas to provide sustained delivery of the compound." The Examiner states that "Mathiowitz et al. disclose the production and use of protein microspheres essentially as Bernstein et al."

According to the Examiner, "Won et al. disclose topically applying porous polymer microspheres containing an active substance to skin to provide controlled release of the active substance for prolonged activity on the skin."

The Examiner concludes that "it would have been obvious to provide the active agent of Richardson et al. in a protein microsphere and use transglutaminase to attach the protein microsphere to skin to provide release of the active agent at a desired rate as suggested by Bernstein et al. or Mathiowitz et al. and Won using protein or polymer microspheres to deliver an active agent at a desired release rate to a site such as skin." The Examiner states that "it would have been expected that transglutaminase will crosslink the glutamine of the protein microspheres with glutamine and/or amino groups of skin since it is known to crosslink protein with transglutaminase." The Examiner further states that "when desiring glutamine groups for reacting with transglutaminase, it would have been obvious to omit treating the protein of the microspheres with transglutaminase for crosslinking to increase stability as may be carried out by Bernstein et al. or Mathiowitz et al. The Examiner also states that "a composition as required by claims 123-125, 135 and 136 would have been obvious from the references since it would have been obvious to provide the protein microspheres with sufficient surface glutamine groups to attach the microspheres to skin with transglutaminase." With respect to claims 143 and 144, the Examiner states that "it would have been obvious to use a non-nucleic acid active agent in the protein microsphere when the function of such an agent is desired, and selecting a preferred particle size within the ranges of Bernstein et al. or Mathiowitz et al. would have required only limited routine experimentation and been obvious. With respect to the kit claims 51 and 75-77, the Examiner states that it "would have been obvious in view of Richardson et al. disclosing providing a package containing an active agent and transglutaminase." The Examiner concludes that "the limitations of dependent claims would have been matters of obvious choice within the ordinary skill of the art in view of the disclosures of the references."

Applicants respectfully traverse the rejection for the reasons stated below.

The primary reference, Richardson et al., teaches attachment of active agents to body tissues using exogenous transglutaminase. The reference demonstrates poor binding of active agents in the absence of exogenous transglutaminase. Richardson et al. further teaches that suitable active agents are those that inherently contain alkylamines or those that can be modified to contain alkylamines. According to the formula provided by Richardson et al., the alkylamine groups are individually conjugated to an active agent and are not directly conjugated to each other. Accordingly, the alkylamine modifications taught by Richardson et al. are not polymers of alkylamines, nor are they polymers containing lysines in either contiguous or non-contiguous form. As an example, the reference demonstrates attachment to human cadaver skin of amine-modified fluorescent latex beads having a diameter of 0.2 μm in the presence of exogenous transglutaminase. The reference stresses the criticality of the amine groups in the active agent for binding to a body tissue.

Richardson et al. does not teach modification of agents with glutamines, instead stressing the criticality of alkylamines as the required reactive group for transglutaminase. The reference also does not teach nor does it contemplate the utility of endogenous transglutaminase in attaching compounds to skin, and thus it cannot teach "sufficient amounts" of transglutaminase amine reactive groups for attachment to a body tissue via endogenous transglutaminase. Finally, Richardson et al. does not teach microparticle attachment as a method for delivering active agent to skin, as the fluorescent spheres of Richardson et al. were used as markers rather than drug carriers.

Accordingly, Richardson fails to teach a number of the elements of the pending claims. For example, Richardson fails to teach microparticles having transglutaminase substrate reactive groups in an amount sufficient to be attached to a body tissue such as a skin surface in the presence of endogenous transglutaminase (claims 1 and 51); microparticles having surface available transglutaminase substrate reactive groups that are glutamines (claim 3); microparticles that are 20 - 35 nm in size (claim 15); microparticles in which the transglutaminase substrate reactive groups are part of a polymer (claim 18) that may be attached to the microparticle (claim 19); a polymer containing transglutaminase surface reactive groups that is composed of at least 50% glutamines (claim 23), or is glutamine-rich at a surface available terminus (claim 24); a method for attaching nonlabeling microparticles to a skin surface (claim 26); nonlabeling microparticles (claims 26 and 76); microparticles containing glutamine-rich polymers (claims 123-125, 135, 136); and microparticles comprising a nonlabeling active agent (claims 144). The Examiner acknowledges that neither Richardson et al. nor any of the secondary references teach the subject matter of claims 22 and 25.

The secondary references of Bernstein et al., Mathiowitz et al., and Won either do not cure the deficiencies of the primary reference or their combination with the primary reference is improper in view of the teachings of the references.

The secondary references of Bernstein et al. and Mathiowitz et al., which provide virtually identical specifications, teach biodegradable protein microspheres preferably made from prolamines (i.e., proteins having large numbers of hydrophobic amino acids such as glutamine, asparagine and proline). These microspheres can be administered topically and can contain enzymes, pesticides and diagnostic agents. As mentioned above (see Rejection under 35 U.S.C § 102(b)), there is no evidence that the microspheres of Bernstein et al. and Mathiowitz et al. possess surface available transglutaminase substrate reactive groups following their manufacture, or that such groups (even if available and accessible to transglutaminase at the surface) are present in amounts sufficient to attach the microparticles to a body tissue such as a skin surface either in the presence of endogenous or exogenous transglutaminase. Moreover, there is no teaching in either Bernstein et al. or Mathiowitz et al. that the microparticles should be covalently attached to a body tissue, such as a skin surface, using transglutaminase.

Notwithstanding this, however, the combination of Richardson et al. and Mathiowitz et al. is improper because Richardson et al. explicitly stresses the criticality of amine groups, rather than carboxamide groups (such as those of glutamine) as suitable reactive substrates for transglutaminase. Accordingly, there is no motivation to combine the Richardson et al. and Bernstein et al. or Mathiowitz et al. references, but rather a "teach away" of such combination because Richardson et al. disclaims the utility of glutamine reactive groups as a way of modifying active agents and/or fluorescent spheres. Moreover, in view of this clear "teaching away" of the use of glutamine reactive groups as modifiers of an active agent by the primary reference, one of ordinary skill would not have had a reasonable expectation of success that the glutamine containing microspheres of Bernstein et al. and Mathiowitz et al. would attach to a body tissue such as skin in the presence of exogenous transglutaminase. Finally, even if such combination could be made, and Applicants maintain that it cannot, the combination still does not teach or suggest all the pending claim limitations. Specifically, the combination fails to teach microparticles, particularly nonlabeling microparticles, having surface available transglutaminase substrate reactive groups present in an amount sufficient to attach the microparticle to a skin surface in the presence of either endogenous or exogenous transglutaminase.

The remaining secondary reference, Won, teaches non-biodegradable microspheres ranging in size from 5 μm to 100 μm that can be used topically. Won teaches that, when used topically, the particles are to be "rubbed into" the skin, rather than covalently attached thereto using transglutaminase. Furthermore, Won teaches that the microspheres should be free from reactive functionalities such as reactive amines in order to prevent interaction with loaded active agents. The teachings of Richardson et

al. and Won are therefore inconsistent as Richardson et al. requires reactive amine groups that act as substrates for transglutaminase, while Won teaches away from the use of particles possessing such reactive amines in order to preserve the integrity and activity of the active agent. Accordingly, the combination of Richardson et al. and Won is also improper in view of this "teaching away." In addition, one of ordinary skill in the art would not have had a reasonable expectation of success that the microspheres of Won could be attached to skin using the method of Richardson et al. because microparticles lacking reactive amines (as taught by Won) could not attach to skin in the presence of exogenous transglutaminase (as taught by Richardson et al.). Even so, if the combination of these references was made, it would still not teach or suggest all the pending claim limitations. In particular, the combination does not teach or suggest the utility of microparticles having surface available reactive groups for attachment to a body tissue such as skin using either endogenous or exogenous transglutaminase.

The combination of Richardson et al. with Bernstein et al. or Mathiowitz et al., either taken with Won is improper for the reasons stated above. Accordingly, the Examiner has failed to establish a *prima facie* case of obviousness in view of these references.

Rejection in view of Richardson et al., Bernstein et al., Mathiowitz et al., Won and Zheng et al.

The Examiner has rejected claims 2, 20, 21, 102 and 117-119 under 35 U.S.C. §103(a) as being unpatentable over the above reference and further in view of Zheng et al.

According to the Examiner, "Zheng et al. disclose producing microspheres containing lysine amino groups to covalently link the microspheres to desired molecules ... (and) ... the microspheres are a blend of a poly(lactide-co-glycolide) and poly (ε CBZ-L-lysine)." The Examiner states that "it would have been obvious to provide the protein microspheres with lysine groups by blending the protein of the microspheres with poly (ε CBZ-L-lysine) as suggested by Zheng et al. since Richardson et al. disclose reacting alkylamine groups with transglutaminase to provide attachment of an active agent to skin."

Applicants respectfully traverse the rejection for the reasons stated below.

Applicants have addressed the obviousness rejection in view of Richardson et al., and Bernstein et al. or Mathiowitz et al., either in further view of Won. The combination of these references is improper given the lack of motivation to combine such references, the lack of reasonable expectation of success for such combination, and the failure of the combination to teach or suggest all of the pending claim limitations.

The Zheng et al. reference teaches microspheres having surface available lysine groups to which a variety of biologically active molecules can be attached. The in vivo properties of these microspheres can be altered based on the nature of the active agents attached to their surface. The reference teaches

microspheres having a diameter in the range of 7 –130 μm, and it further demonstrates that, following treatment with lithium and ammonium, 2.3% of total microsphere amino groups were deprotected.

The teachings of the Zheng et al. reference are inconsistent with those of Richardson et al. In particular, Zheng et al. teaches that reactive amines on the surface of microparticles are to be used to attach active agents or targeting molecules for delivery to specific sites in the body. Once so modified, the microparticles would no longer be a suitable substrate for transglutaminase activity as taught by Richardson et al. The Zheng et al. reference does not contemplate that the microparticles can be directly attached to a body tissue via the reactive amine groups, and accordingly, it fails to teach that the surface reactive lysines must be present in amounts sufficient to attach the microparticle to a body tissue such as skin in the presence of endogenous or exogenous transglutaminase. There is no motivation to combine the references, and moreover there is no reasonable expectation of success in using the microspheres of Zheng et al. in the method of Richardson et al.

Finally, the reference does not teach several elements of the pending claims, and thus cannot cure the deficiencies of Richardson et al. Thus, even if the teachings of Richardson et al. and Zheng et al. could be combined in spite of the inconsistency regarding further modification of the surface available lysine groups, the combination still fails to teach the pending claim limitations including microparticles having transglutaminase substrate reactive groups in an amount sufficient to be attached to a body tissue such as a skin surface in the presence of endogenous transglutaminase, and methods related thereto (claims 1 and 51); microparticles having surface available transglutaminase substrate reactive groups that are glutamines (claim 3); microparticles that are 20 nm to 35 nm in size (claim 15); microparticles in which the transglutaminase substrate reactive groups are part of a polymer that may be attached to the microparticle (claims 18 and 19); a polymer containing transglutaminase surface reactive groups that is composed of at least 50% glutamines, or is glutamine-rich at a surface available terminus (claims 23 and 24); a method for attaching nonlabeling microparticles to a skin surface (claim 26); nonlabeling microparticles (claims 26 and 76); microparticles containing glutamine-rich polymers (claims 123-125, 135 and 136); and microparticles comprising a nonlabeling active agent (claim 144). Accordingly, the Examiner has failed to make a *prima facie* case of obviousness over the art of record.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejections under 35 U.S.C. §103(a).

Rejection under the Judicially Created Doctrine of Obviousness-Type Double Patenting

The Examiner has rejected claims 1-26, 51, 75-77, 102, 117-119, 123-125, 135, 136, 143 and 144 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over

claims 1-48 of U.S. Patent No. 6,267,957 in view of Bernstein et al. or Mathiowitz et al. and each taken with Won.

The Examiner states that "the claims of the patent require attaching an agent to body tissue by applying to the body tissue a conjugate of the agent and a linking molecule such as a polymer containing glutamines or lysines in the presence of transglutaminase to crosslink the conjugate to the body tissue via the linking molecule." According to the Examiner, "it would have been obvious to substitute the conjugate containing an agent in the patent claims with protein microspheres containing the agent as suggested by Bernstein et al. or Mathiowitz et al. and Won."

Applicants respectfully traverse the rejection for the reasons set forth below.

The claims in US 6,267,957 are directed to methods for using conjugates of agents and linking molecules containing either two linked glutamines or three linked lysines. In some aspects, the conjugates are preformed and applied to a body tissue, while in others, the linking molecule is first attached to the body tissue followed by attachment thereto of the agent. The claims do not recite microparticles.

The pending claims relate to compositions and methods of use relating to microparticles having surface available transglutaminase substrate reactive groups in amounts sufficient to attach the microparticle to a body tissue. Microparticles are a patentably distinct species of agents that can be attached to a body tissue. Accordingly, the double patenting rejection is considered improper as the scope and content of the patent claim is patentably distinct from the scope and content of the application claims as pending.

Notwithstanding this, the combination of Bernstein et al. or Mathiowitz et al. and Won (all of which have been discussed above) results in prolamine containing microspheres that lack surface reactive groups because of the teaching by Won that reactive groups should be removed to preserve integrity of a loaded active agent. These references either alone or in combination do not teach that particles can be attached to skin using transglutaminase, and thus cannot be combined with US 6,267,957.

Accordingly, the pending claims are patentably distinct from the issued patent claims, either when taken alone or in combination with the prior art.

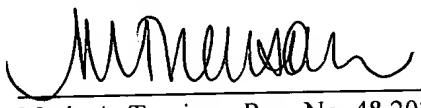
In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw the rejection under the judicially created doctrine of obviousness-type double patenting.

Summary

Applicants believe that each of the pending claims is now in condition for allowance. Applicants respectfully request that the Examiner telephone the undersigned in the event that the claims are not found to be in condition for allowance.

If the Examiner has any questions and believes that a telephone conference with Applicants' agent would prove helpful in expediting the prosecution of this application, the Examiner is urged to call the undersigned at (617) 720-3500 (extension 266).

Respectfully submitted,



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Attorney's Docket No.: H0535/7012 (ERG/MAT)
DATE: February 11, 2002
x02/10/02

APPENDIX A

MARKED-UP SPECIFICATION

Please re-write the specification as indicated below.

Please re-write the paragraph beginning on page 6, line 30 as follows:

In certain embodiments, the active agent is selected from the group consisting of a cosmetic agent, a bulking agent, a hair conditioning agent, a hair fixative, a sunscreen agent, a moisturizing agent, a depilatory agent, an anti-nerve gas agent, a film forming agent, a vitamin, an insect repellant, a coloring agent, a pharmaceutical agent, a ligand-receptor complex and a receptor of a ligand-receptor complex. In one embodiment, the active agent comprised within the microparticle is not itself [be] a substrate of transglutaminase. In another embodiment, the active agent is a non-protein active agent. In yet another embodiment, the active agent is a non-nucleic acid active agent.

Please re-write the paragraph beginning on page 21, line 14 as follows:

Encapsulated microspheres made from poly(lactide-co-glycolide) and poly(ε-CBZ-L-lysine) and subsequently treated so as to expose surface reactive amino groups have been reported previously. (Zheng and Hornsby, 1999, Biotechnol. Prog. 15:763-767) Once the microspheres are formed using double-emulsification/solvent evaporation (Alonso, et al., 1993, Pharmacol. Res. 10:945-953), the carbobenzoxy (i.e., CBZ) protective groups are removed using either acid hydrolysis or lithium/liquid ammonia reduction, thereby exposing reactive amine groups. Lithium/liquid ammonia reduction is recommended if microspheres are desired, given its less harsh effect of the external surface of the microparticle. In addition, the lithium treatment was reported to be more effective in producing surface reactive amino groups than was the acid hydrolysis procedure. If a solid surface particle (i.e., a microsphere) is desired, the lithium treatment may be preferred. In this latter method, the active agent may be added during the formation of the microparticles since the lithium treatment reportedly does not create pores in the surface of the particles and thus will not adversely affect the agent. If, on the other hand, a surface porous particle is desired, then the acid hydrolysis method may be preferred, provided the agent is either resistant to the acid treatment or is loaded into the particles following acid treatment.

MARKED-UP CLAIMS

Please re-write the claims as indicated below.

1. (Amended) A method of treating a subject to attach microparticles to a skin surface containing endogenous transglutaminase of the subject comprising

contacting the skin surface containing endogenous transglutaminase with microparticles having surface available transglutaminase substrate reactive groups in an amount sufficient to attach the microparticles to the skin surface in the presence of the endogenous transglutaminase,

allowing the microparticles to remain in contact with the skin surface for a time sufficient to permit a layer of microparticles to covalently attach to the skin surface.

2. The method of claim 1, wherein the surface available transglutaminase substrate reactive groups are lysines.

3. The method of claim 1, wherein the surface available transglutaminase substrate reactive groups are glutamines.

4. The method of claim 1, wherein the layer of microparticles is non-planar.

5. The method of claim 1, wherein the microparticles further comprise an active agent.

6. The method of claim 5, wherein the active agent is a non-nucleic acid active agent.

7. The method of claim 5, wherein the active agent is a non-protein active agent.

8. The method of claim 5, wherein the active agent is selected from the group consisting of a cosmetic agent, a bulking agent, a hair conditioning agent, a hair fixative, a sunscreen agent, a moisturizing agent, a depilatory agent, an anti-nerve gas agent, a film forming agent, a vitamin, an insect repellant, a coloring agent, a pharmaceutical agent, a ligand-receptor complex and a receptor of a ligand-receptor complex.

9. The method of claim 5, wherein the active agent is not itself a substrate of transglutaminase.

10. The method of claim 1, wherein the microparticles further comprise a synthetic polymer.

11. The method of claim 10, wherein the synthetic polymer is latex.

12. The method of claim 10, wherein the synthetic polymer is polystyrene.

13. The method of claim 1, wherein the microparticles are porous.

14. The method of claim 1, wherein the microparticles are 100 nm to 500 nm in size.

15. The method of claim 1, wherein the microparticles are 20 nm to 35 nm in size.

16. The method of claim 1, wherein the microparticles are non-biodegradable.

17. The method of claim 1, wherein the microparticles are detergent insoluble.

18. The method of claim 1, wherein the transglutaminase substrate reactive groups are part of a polymer.

19. The method of claim 18, wherein the polymer is covalently attached to the microparticle.

20. The method of claim 18, wherein the polymer is comprised of at least 50% lysines.

21. The method of claim 18, wherein the polymer is lysine-rich at a surface available terminus.

22. (Amended) The method of claim 18, wherein the polymer comprises a polymer selected from the group consisting of <u>polymers containing</u>:

(a) at least two contiguous linked lysines,

(b) at least three contiguous linked lysines,

(c) at least four contiguous linked lysines, and

(d) at least five contiguous linked lysines.

23. The method of claim 18, wherein the polymer is comprised of at least 50% glutamines.

24. The method of claim 18, wherein the polymer is glutamine-rich at a surface available terminus.

25. (Amended) The method of claim 18, wherein the polymer comprises a polymer selected from the group consisting of polymers containing:

(a) at least five contiguous linked glutamines,

(b) at least ten contiguous linked glutamines,

(c) at least fifteen contiguous linked glutamines, and

(d) at least twenty contiguous linked glutamines.

26. (Amended) A method of treating a subject to attach nonlabeling microparticles to a skin surface of the subject comprising

contacting the skin surface with nonlabeling microparticles having surface available transglutaminase substrate reactive groups in an amount sufficient to attach the nonlabeling microparticles to the skin surface in the presence of exogenous transglutaminase,

applying exogenous transglutaminase to the skin surface, and

allowing the nonlabeling microparticles and exogenous transglutaminase to remain in contact with the skin surface for a time sufficient to permit a layer of nonlabeling microparticles to covalently attach to the skin surface.

51. A kit comprising

a microparticle comprising surface available transglutaminase substrate reactive groups in an amount sufficient to attach the microparticle to a skin surface in the presence of endogenous transglutaminase, and

instructions for topically administering the microparticle to a skin surface.

75. The kit of claim 51, wherein the microparticle is provided in a topically administered form selected from the group consisting of an ointment, an aerosol, a gel, and a lotion.

76. (Amended) A kit comprising

a nonlabeling microparticle having surface available transglutaminase substrate reactive groups in an amount sufficient to attach the nonlabeling microparticle to a skin surface in the presence of exogenous transglutaminase, and

instructions for topically administering the nonlabeling microparticle and transglutaminase to a skin surface.

77. The kit of claim 76, wherein the kit further comprises transglutaminase.

102. A composition comprising

a microparticle comprising an active agent and a lysine-rich polymer having transglutaminase substrate reactive groups, wherein the microparticle is non-biodegradable, and the transglutaminase substrate reactive groups are surface available.

117. The composition of claim 102, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of endogenous transglutaminase.

118. The composition of claim 102, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of exogenous transglutaminase.

119. The composition of claim 102, wherein the lysine-rich polymer comprises a polymer of amino acids and wherein at least 50% of the amino acids are lysine.

123. A composition comprising

a microparticle comprising an active agent and a glutamine-rich polymer having transglutaminase substrate reactive groups, wherein the transglutaminase substrate reactive groups are surface available.

124. The composition of claim 123, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of endogenous transglutaminase.

125. The composition of claim 123, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of exogenous transglutaminase.

135. (Amended) The composition of claim [131] 123, wherein the glutamine-rich polymer is covalently linked to the synthetic polymer.

136. The composition of claim 123, wherein the glutamine-rich polymer comprises a polymer of amino acids and wherein at least 20% of the amino acids are glutamines.

[144] 143. (Amended) A composition comprising

a microparticle comprising a non-nucleic acid nonlabeling active agent, and

covalently attached surface available transglutaminase substrate reactive groups,

wherein the microparticle is 100 nm to 500 nm in size.

[145] 144. (Amended) The composition of claim [144] 143, wherein the surface available transglutaminase substrate reactive groups are free pendant groups.